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### Clinical and genetic spectrum of hereditary cardiac arrhythmia syndromes

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# 14

## **Desmoglein-2 and Desmocollin-2 Mutations in Dutch Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy patients: Results from a multicenter study**

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## Abstract

### Background

This study aimed to evaluate the prevalence and type of mutations in the major desmosomal genes, Plakophilin-2 (PKP2), Desmoglein-2 (DSG2) and Desmocollin-2 (DSC2), in Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy (ARVD/C). We also aimed to distinguish relevant clinical and ECG parameters.

### Methods & Results

Clinical evaluation was performed according to the Task Force Criteria (TFC). We analyzed the genes in: (a) 57 patients who fulfilled the ARVD/C TFC (TFC+), (b) 28 patients with probable ARVD/C (1 major and 1 minor, or 3 minor criteria) and (c) 31 patients with 2 minor or 1 major criteria. In the TFC+ ARVD/C group, 23 patients (40%) had PKP2 mutations, 4 (7%) had DSG2 mutations, 1 patient (2%) carried a mutation in DSC2, while 1 patient (2%) had a mutation in both DSG2 and DSC2. Among the DSG2 and/or DSC2 mutation-positive TFC+ ARVD/C probands, 2 carried compound heterozygous mutations and 1 had digenic mutations. In probable ARVD/C patients and those with 2 minor or 1 major criteria for ARVD/C, mutations were less frequent and they were all heterozygous. Negative T waves in the precordial leads were observed more ( $p < .002$ ) among mutation carriers than non-carriers, and in particular in PKP2 mutation carriers.

### Conclusions

Mutations in DSG2 and DSC2 are together less prevalent (10%) than PKP2 mutations (40%) in Dutch TFC+ ARVD/C patients. Interestingly, bi-allelic or digenic DSC2 and/or DSG2 mutations are frequently identified in TFC+ ARVD/C patients, suggesting that a single mutation is less likely to cause a full blown ARVD/C phenotype. Negative T waves on ECG were prevalent among mutation carriers ( $p < .002$ ).

**Key Words:** Arrhythmia • Cardiomyopathy • Desmosomes • Genetics

## Introduction

Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy (ARVD/C) is a familial disease characterized by progressive fibrofatty replacement of the right ventricular (RV) myocardium.<sup>1</sup> The main histological feature is progressive loss of RV myocardium, which is replaced with adipose and fibrous tissue. These changes may be localized and in the early stages are often confined to the so-called “triangle of dysplasia”: the inflow, outflow, and apical regions of the RV.<sup>1</sup> Aneurysm formation is typical. Diffuse myocardial involvement leads to global RV dilation. Left ventricular (LV) involvement occurs with disease progression and was present on histology in >75% of cases in a multicenter pathological study.<sup>2</sup> ARVD/C is a genetically heterogeneous disease and is most commonly inherited as an autosomal-dominant trait with incomplete penetrance and variable expression. Its clinical picture is mainly characterized by the occurrence of ventricular arrhythmias and related consequences.<sup>3-4</sup> Estimates of the prevalence of ARVD/C in the general population range from 1 in 2,000 to 1 in 5,000; it affects men more frequently than women, with an approximate gender ratio of 3:1.<sup>3-4</sup> A familial background has been demonstrated in 30-50% of ARVD/C cases<sup>3-5</sup> and the majority of disease-causing mutations have so far been identified in genes encoding proteins of specialized adhesive junctions between cells, also known as desmosomes.<sup>5-18</sup> Mutations in the PKP2 gene encoding Plakophilin-2 are the most prevalent and were identified in up to 43% of unrelated ARVD/C index patients.<sup>5,7,10</sup> Dominant mutations in genes encoding Desmoglein-2 (DSG2) and Desmocollin-2 (DSC2) have been reported in up to 12% and 5% of ARVD/C patients, respectively.<sup>12,16-17,19-20</sup> Rarely, dominant mutations in the Plakoglobin (JUP), Desmoplakin (DSP) and TMEM43 genes have been reported in patients with ARVD/C, with recessive mutations in JUP and DSP being causal for the cardiocutaneous diseases, Naxos disease and Carvajal syndrome, respectively.<sup>6,8,21-24</sup> This study is an extension of our previous study in which we elucidated PKP2 mutations in our Dutch ARVD/C cohorts.<sup>5</sup> Our goal now was to evaluate the prevalence of mutations in three cardiac desmosomal genes, PKP2, DSG2 and DSC2, and to study their impact on clinical phenotypes in three groups: (a) patients fulfilling the ARVD/C task force criteria (TFC+),<sup>25</sup> (b) patients with probable ARVD/C (1 major and 1 minor, or 3 minor criteria), and (c) patients with either 2 minor or 1 major criteria for ARVD/C.<sup>25</sup> Genotype-phenotype analysis, of both clinical and ECG features, was also performed to identify potentially distinguishing features.

## Methods

## Clinical evaluation and diagnostic criteria

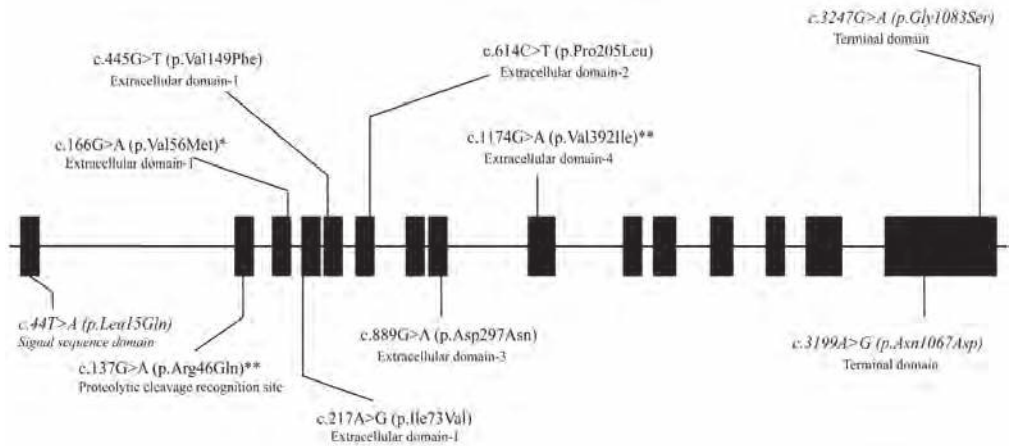
We evaluated 116 white, unrelated, index patients in four university hospitals in the Netherlands. A case history was taken from all patients, and they were physically examined and evaluated by 12-lead ECG, 24-hour Holter monitoring, exercise testing, and 2-dimensional transthoracic echocardiography. Diagnosis of ARVD/C was based on the diagnostic criteria of the Task Force of the European Society of Cardiology/International Society and Federation of Cardiology.<sup>25</sup> (Table 1). In cases of doubtful diagnosis, patients were discussed with experienced cardiologists from the four centers in a consensus meeting. The 116 index patients were subdivided into: (a) 57 TFC+ patients, (b) 28 probable ARVD/C (who had 3 minor criteria, or 1 major and 1 minor criteria) and (c) 31 patients with 2 minor or 1 major criteria of ARVD/C (not including family history). 8 TFC+ patients from our previous study were excluded due to absence of DNA.<sup>5</sup> Instead, 8 new TFC+ patients were included in this study (Table 2, marked 'n'). The onset of disease manifestation was defined as the age at which initial symptoms most likely related to ARVD/C emerged, including paroxysmal tachycardia, prolonged syncope, and successful resuscitation.

## Genetic studies

Genomic DNA was extracted from whole blood or paraffin-embedded tissues according to the established protocol (Qiagen). Detailed procedures for screening the PKP2 gene were described earlier.<sup>5</sup> DSG2 and DSC2 coding regions (exons 1-15 and exons 1-16, respectively) were screened for mutations. Primer sequences and PCR conditions are available on request. Mutational analysis of the amplicons was performed by denaturant high performance liquid chromatography (DHPLC) (Transgenomic Wave) and denaturing gradient gel electrophoresis (DGGE). PCR products with altered DHPLC peak or DGGE variants were purified using QIAquick PCR purification kit (Qiagen) and were sequenced bidirectionally on an ABI 377 sequencer.

In this study, the pathogenic nature of the identified missense mutations were judged on the basis of four criteria (for an overview of criteria potentially useful for classifying variants see Goldgar et al.<sup>26</sup>): (1) the differences in physico-chemical properties of the amino acids involved in the respective substitutions, (2) the evolutionary conservation of the amino acids across several species, (3) the presence in an evolutionary conserved region, and (4) the localization within a functionally important domain (predicted or unpredicted). Missense mutations were considered pathogenic when the respective amino acid substitutions satisfied two or more of these criteria. Amino acid substitutions were considered unclassified variants (UVs) when these satisfied none or only one of the criteria mentioned above and are not known as single nucleotide polymorphisms. In all cases, the reported mutations/ variations were not identified in at least 150 ethnically-matched control persons. Notably, in

some cases the mutations had been reported in previous studies in ARVD/C patients.



**Figure 1a.** Schematic representation of the DSG2 mutations and unclassified variants (UVs). Most mutations except three (indicated by \*) are previously unreported.<sup>12,17</sup> \*\* indicates the mutation was detected in two unrelated patients/families and was also reported previously. UVs are shown in italics.

## Statistical analysis

Clinical characteristics in the patients with a PKP2, DSG2 or DSC2 mutation/UV were compared using Fisher's exact test. Values of  $P < 0.05$  were considered significant. All data were analyzed with the Statistical Package for Social Sciences (SPSS version 15.0; SPSS, Inc., Chicago, IL).

## Results

### Clinical evaluation

Clinical data of the 57 TFC+ and 28 probable ARVD/C patients are shown in Tables 2 and 3, respectively. The average age of first clinical presentation was 33 years in PKP2, 36 years in DSG2, 42 years in DSC2, and 14 years for combined DSC2/DSG2 mutation carriers and 37 years for non-mutation carriers (range 14-68 yrs); these ages did not differ significantly between men and women.

### Genetic studies

We screened the PKP2, DSG2 and DSC2 genes in 116 patients.

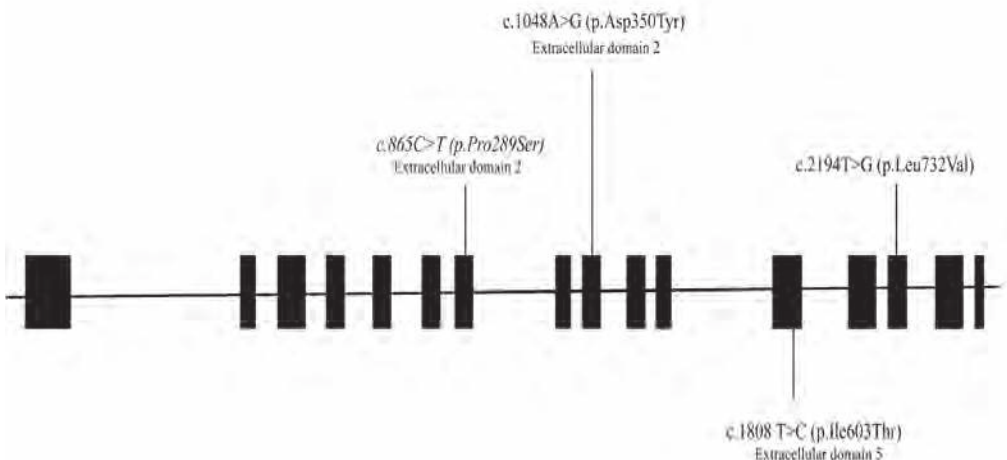
### TFC+ group

Of 57 TFC+ ARVD/C patients, most of whom were included in our previous study<sup>5</sup>, 23 carried a mutation in PKP2 (Table 2; Figure 1c). Four TFC+ patients had mutations in DSG2 (Figure 1a and 1c and Table 2); two of them (patients-25 and -41 had mutations (and unclassified variants (UVs) in both alleles. Patient-25 carried compound heterozygous, p.Pro205Leu mutation and an UV p.Gly1083Ser in DSG2. Patient-41 was homozygous for p.Asp297Asn in DSG2.

Patients-39 (female) and -52 (male) had an identical mutation p.Arg46Gln in DSG2 (Table 2). In addition they carried p.Val158Gly (DSG2) on the same allele, which has been described as a mutation in a UK study.<sup>17</sup> In our study, we also found this p.Val158Gly in our control population, and similar results were recently reported by Posch et al.<sup>27</sup>, thus we should consider p.Val158Gly as a non-synonymous SNP.

Patient-24, a 56-year old male, was the only patient in this group who had a heterozygous mutation p.Ile603Thr only in DSC2 (Table 2 and Figure 1b). Patient-40 carried a heterozygous p.Val392Ile mutation in DSG2 and p.Leu732Val mutation in the DSC2 gene (Figure 1a, Table 2). We could not identify any mutations in any of these three genes in 28 (49%) TFC+ patients (Figure 1c). A schematic representation of the mutation yield from the PKP2, DSG2 and DSC2 screening is shown in Figure 1c.

### TFC- group



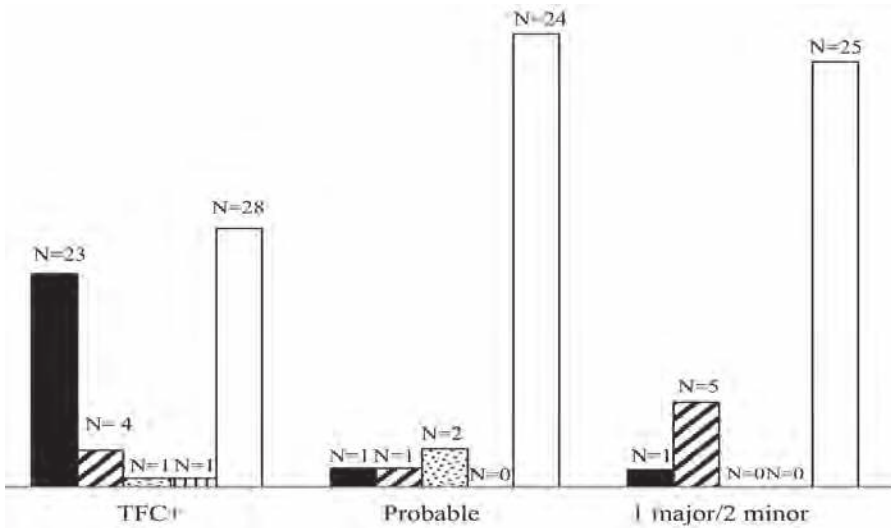
**Figure 1b.** Schematic representation of the DSC2 mutations and UVs. UVs are shown in italics.



	Major	Minor
Global and/or regional dysfunction and structural alterations	Severe RV dilatation and reduction in systolic function with no (or only mild) LV impairment Localized RV aneurysms	Mild global RV dilatation and/or reduced EF with normal LV Regional RV hypokinesia Mild segmental RV dilatation
Tissue characterization of walls	Fibrofatty replacement of myocardium on EMB	
Repolarization abnormalities		Negative T-waves (V2 and V3); >12 years of age in the absence of RBBB
Depolarization abnormalities	Epsilon waves or localized prolongation of the QRS complex in V1-V3 (>110ms)	Late potentials on SA-ECG
Arrhythmias		Sustained and non-sustained VT with LBBB morphology (on ECG, Holter, exercise testing) >1000 ventricular extrasystoles/ 24 hrs
Family history	Familial disease confirmed at necropsy or surgery	SCD (<35 yrs) due to suspected disease in a family member Family history of clinical diagnosis based on present criteria

RV right ventricle, LV left ventricle, EF ejection fraction, EMB endomyocardial biopsy, RBBB right bundle branch block, LBBB left bundle branch block, VT ventricular tachycardia, SCD sudden cardiac death

**Table 1.** Task force criteria for the diagnosis of Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy. Either 4 minor criteria, 2 major, or 1 major + 2 minor criteria are sufficient to make a diagnosis of ARVC.<sup>25</sup> EF=ejection fraction, EMB= endomyocardial biopsy, LBB= left bundle branch, LV=left ventricular, RV=right ventricular, SCD=sudden cardiac death, VT= ventricular tachycardia.



**Figure 1c:** Graphical representation of *PKP2*, *DSG2*, *DSC2* gene screening in the ARVD/C, probable ARVD/C, and patients with attenuated features of ARVD/C. Filled bars represent TFC+ patients, slashed bars represent probable ARVD/C, and dotted bars represent patients with attenuated features of ARVD/C. The x-axis represents analyzed genes and the y-axis represents percentages of mutations identified in each group. On top of each bar, the number of mutation carriers or non-mutation carriers of the respective genes are represented.

Index patient	Gender	Age initial presentation	Structural alteration	Tissue characterization	Repolarization abnormalities			Depolarization/conduction abnormalities			Arrhythmias			PKP2	DSG2	DSC2
					Negative T wave	Epsilon wave	Late potential	LBBVT	VES	Family history						
P-1	m	35	++		+		+	+		++			c.1211_1212insT frameshift			
P-2	m	24	++		+	++		+								
P-3	m	32	++		+			+					c.148_151delACAG frameshift			
P-4	m	46	++		+	++		+								
P-5	m	21	++							++						
P-6	f	17	++		+					+	++		c.2489+1 G>A frameshift			
P-7	m	17	++		+		+	+					c.2489+1G>A frameshift			
P-8	m	22		++	+			+								
P-9 <sup>a</sup>	f	17			+					+	++					
P-10	f	22		++	+		+	+								
P-11	m	35	++		+					+	+		Trp848X			
P-12	m	30	+	++	+			+					Cys796Arg			
P-13	m	33	++	++	+		+	+			+		Cys796Arg			
P-14	m	58	+	++	+		+	+								
P-15	m	58	+	++	+		+	+								
P-16	f	53			+					+	++		Arg79X			
P-17	f	39	++	++	+			+								
P-18	f	31		++	+		+	+					Tyr616X			
P-19	m	17	++	++	+			+								
P-20	m	46		++	+		+				+					
P-21	m	48	+		+		+	+								
P-22	f	44	++		+			+								
P-23 <sup>a</sup>	m	44	+			++		+								
P-24 <sup>a</sup>	m	56			+	++	+			+					Ile603Thr	
P-25	m	30	++		+	++		+		+				Pro205Leu; Gly1083Ser		
P-26	m	41	++		+	++		+					Tyr86X			
P-27	m	38		++				+		+						
P-28 <sup>a</sup>	m	48	+	++			+	+								
P-29	m	38			+	++		+		+			Trp676X			
P-30 <sup>a</sup>	m	22	++		+					+						
P-31	m	43	+		+	++		+		+			Arg79X			
P-32	f	41			+	++		+								
P-33	m	28	++			++		+								
P-34	f	30	+		+			+		++			Tyr807X			
P-35	m	29	++		+			+		+			2489+4A>C frameshift			
P-36	f	40	++		+			+					Arg79X			
P-37	m	43	++		+			+								
P-38	m	21	+		+			+		++			Cys796Arg			
P-39 <sup>a</sup>	f	25	++		+			+		+				Arg46Gln		
P-40	f	14	++				+	+					Val392Ile	Leu732Val		
P-41	m	36	++		+			+		+			Asp297Asn (homozygous)			
P-42	m	47	+			++	+	+								
P-43	m	48	++			++		+								
P-44	f	58	+			++		+								
P-45	f	50	++					+		+			Gln133X			
P-46	m	29	++		+			+					Arg79X			
P-47	f	37	+	++	+			+					Cys796Arg			
P-48	m	39	++		+			+					Arg264Asn			
P-49	f	42			+		+	+		+			Ser688Pro			
P-50 <sup>a</sup>	m	24	++		+	++		+		+						
P-51 <sup>a</sup>	m	38	++		+			+								
P-52	m	33	+		+	+		+		+				Arg46Gln		
P-53	m	28	++		+			+		++			Arg79X			
P-54	f	40	++		+	++		+					Cys796Arg			
P-55	m	12	++		+			+								
P-56	m	22	++		+			+		+			Arg79X			
P-57	m	34	++		+	++		+								

m male, f female, LBBVT left bundle branch block ventricular tachycardia, VES ventricular extrasystoles

**Table 2.** Clinical characteristics of the probands with TFC+ ARVD/C patients.

++ indicates a major criterion; + minor criterion; f, female; m, male. Mutations identified in the PKP2, DSG2 and DSC2 are indicated. UVs are shown in italics.

### Probable ARVD/C patients

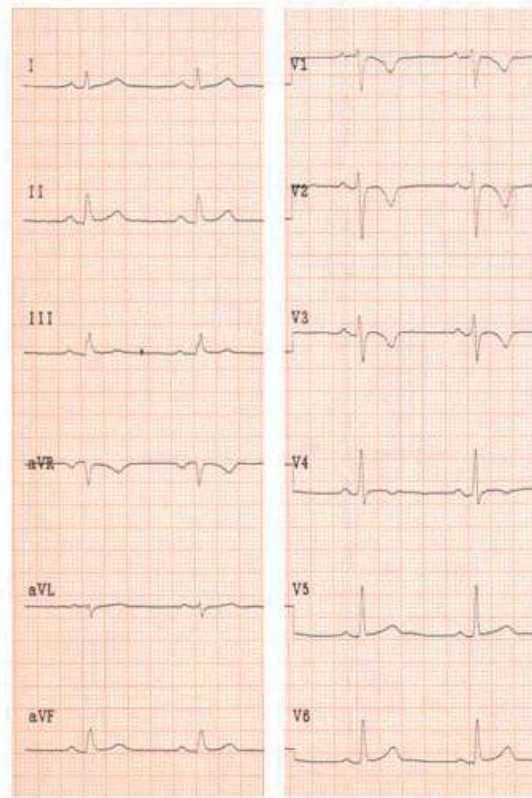
In the patients with probable ARVD/C (n=28), we detected one heterozygous truncating mutation c.397C>T (p.Gln133X) in PKP2 (patient-63; Table 3). Patient-59 had a heterozygous UV p.Leu15Gln in DSG2 (Figure 1a, and Table 3). Leu15 in DSG2 is not very well conserved across diverse species, but it is located in a functionally important signal sequence domain. Patient-64 carried a heterozygous mutation p.Asp350Tyr and Patient-75 carried an UV p.Pro289Ser, in DSC2 (Figure 1b and Table 3). We were able to study the segregation of the mutation in the family of patient-64: it was also found in a brother and a sister, with a positive signal averaged-ECG as a sole manifestation, at ages 48 and 47 years respectively.

Index patient	Gender	Age at initial presentation (yrs)	Structural alteration	Tissue characterisation	Repolarization abnormalities	Negative T wave	Depolarization/ conduction abnormalities	Epsilon wave	Late potentials	Family history	Arrhythmias	LBBVT	VES	PKP2	DSG2	DSC2
P-58	m	42	+					++								
P-59	m	55						++				+			<i>Leu15Gln</i>	
P-60	m	48		++							+					
P-61	f	26			+			+			+					
P-62	m	19						++		+						
P-63	f	25	+		+						+			Gln133X		
P-64	f	48		++							+					Asp350Tyr
P-65	m	39	++	++							+					
P-66	f	37								+						
P-67	f	40						++			+					
P-68	m	68						++			+					
P-69	f	51						++			+					
P-70	m	29	+	++												
P-71	m	48	++							+						
P-72	m	52	+						+			+				
P-73	f	28	++								+					
P-74	m	29	+		+						+					
P-75	m	21	++		+											<i>Pro289Ser</i>
P-76	f	40	++								+					
P-77	f	47	++								+					
P-78	m	25	++								+					
P-79	f	20	+							++						
P-80	m	55	++								+					
P-81	f	41						++			+					
P-82	m	61	+		+							+				
P-83	m	42	+		+						+	+				
P-84	f	42			+						+	+				
P-85	F	17			+					+		+				

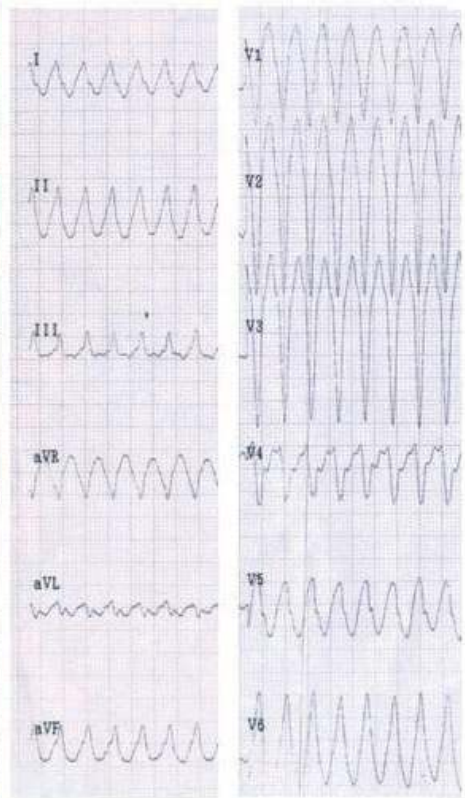
m: male, f female, LBBVT: left bundle branch ventricular tachycardias, VES: ventricular extrasystoles

**Table 3.** Clinical characteristics of the probable ARVD/C patients.

++ indicates a major criterion; + minor criterion; f, female; m, male. Mutations identified in the PKP2, DSG2 and DSC2 are indicated. UVs are shown in italics.



**Figure 2a:** ECGs of a male symptomatic patient at baseline (2a) and during palpitations (2b). At baseline there is a sinus rhythm of 60 bpm, intermediate electrical axis, normal conduction intervals and normal repolarization with exception of the negative T-waves in the precordial leads V1-V4. Fig 2b shows ventricular tachycardia (250 bpm) with left bundle branch morphology and an intermediate electrical axis suggesting its origin in the RV free wall



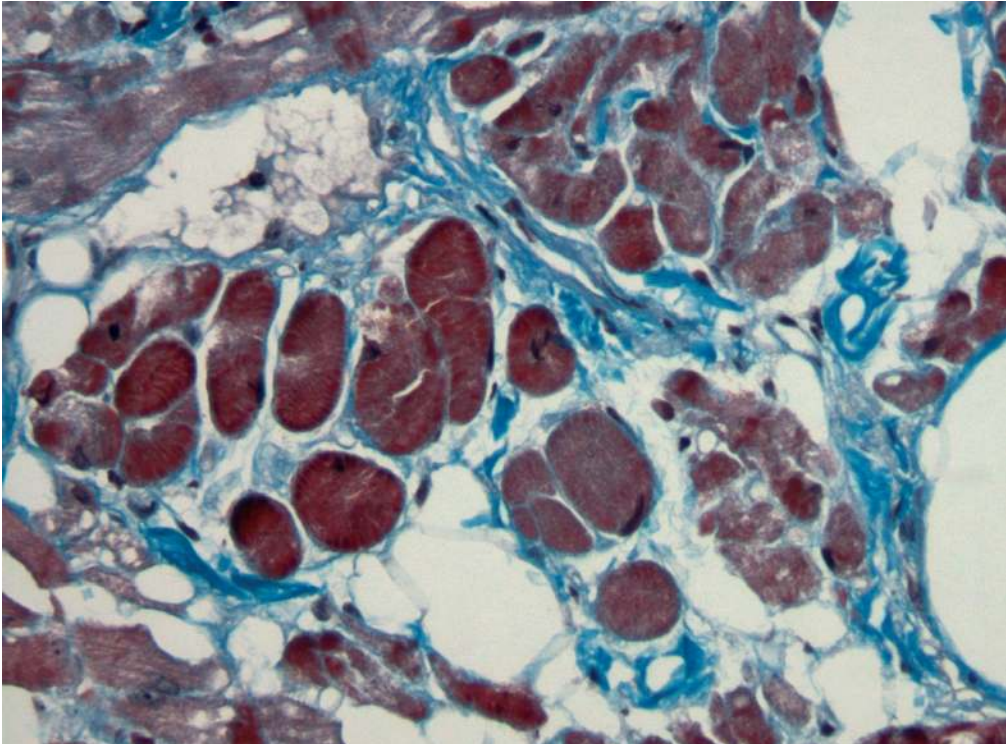
**Figure 2b:** Histology: high powered visualization of fibrolipomatosis surrounding atrophic cardiomyocytes and indicative of ARVD/C. The endomyocardial biopsy was taken from patient-59 who carries the heterozygous p.Asp350Tyr mutation in the DSC2 gene.

### Patients with 1 major or 2 minor criteria for ARVD/C

In this TFC-group of patients (n=31) who had only 1 major or 2 minor criteria of ARVD/C, we detected two UVs in PKP2: c.1592C>T (p.Ile531Ser), which has been reported in the Finnish population as a polymorphism<sup>28</sup>, and c.1012A>G (p.Thr338Ala). In DSG2 five heterozygous mutations (including one UV) were found: p.Val56Met, p.Ile73Val, p.Val149Phe, p.Val392Ile, p.Asn1067Asp (UV),(Figures 1a, 1b).

The patient with p.Val392Ile amino-acid change was a female of 34 years. She had negative T waves at V4-6, a low voltage ECG, was later diagnosed with dilated cardiomyopathy at

the age of 47 years, and experienced an out-of-hospital cardiac arrest due to ventricular fibrillation. She died with RV failure at 56 years and histology showed typical features of ARVD/C in the RV and dilated cardiomyopathy (DCM) with fibrosis in the LV. p.Val392Ile mutation in DSG2 was diagnosed post mortem in paraffin-embedded tissue material. Her sister had died suddenly at the age of 32 years. Her nephew (the dead sister's son) showed negative T-waves in V4-V6 and was diagnosed with DCM at the age of 49 years after experiencing a near-syncope. He was also found to carry this p.Val392Ile DSG2 mutation. These data suggest a left-dominant arrhythmogenic cardiomyopathy.



**Figure 3:** Histology: high powered visualization of fibrolipomatosis surrounding atrophic cardiomyocytes and indicative of ARVD/C. The endomyocardial biopsy was taken from patient-59 who carries the heterozygous p.Asp350Tyr mutation in the DSC2 gene.

### Mutation characteristics

The DSG2 mutations identified in our study, p.Val392Ile, p.Val56Met, and p.Arg46Gln, were previously reported in studies from the USA, UK and Germany.<sup>12,17,27</sup> We identified four novel DSG2 mutations: p.Ile73Val, p.Val149Phe, p.Pro205Leu, and p.Asp297Asn (Figure 1a). In addition, we detected three novel UVs in DSG2 (p.Leu15Gln, p.Asn1067Asp and p.Gly1083Ser) (Figure 1a). In DSC2 we identified three novel mutations, p.Asp350Tyr,



p.Ile603Thr, p.Leu732Val, and one UV, p.Pro289Ser (Figure 1b). The mutations in various groups of patients are shown in Figure 1c.

The DSG2 and DSC2 mutations we identified are all missense mutations (Figures 1a, 1b and Tables 2, 3), in contrast to PKP2 mutations, which are mostly truncating/frameshift mutations.<sup>5</sup> In this study, three patients (patients-25 and -41, TFC+ group; and 1 patient with two minor criteria) among the DSG2 mutation carriers were familial cases. The DSC2 mutation carriers were all sporadic, with no family history pertaining to ARVD/C.

### **Genotype-phenotype relationships/phenotypic characteristics**

Figures 2a and 2b show examples of a classical ARVD/C ECG recorded from a male symptomatic patient in this study. Figure 3 shows a representative ARVD/C histopathology of myocardial tissue taken from patient-64. We have compared various pertinent phenotypic features (age of onset, cardiac structural alteration, ECG features, VES) among the patients with defects in PKP2 (n=24), DSG2 (n=5), DSC2 (n=3), and both DSG2/DSC2 (n=1). Patients without a mutation in any of these three genes were also compared with the mutation carriers and TFC+ and probable ARVD/C patients were included in this analysis.

The presence of T wave inversions in right precordial leads on 12-lead ECG in individuals with a mutation (PKP2, DSG2 and DSC2) was significantly higher than that of individuals without a mutation ( $P < .002$ ). Among the 54 patients with a negative T wave, 26 carried mutation/UVs in one of the three genes. In contrast, only 4 patients (of 31) with a normal, positive T wave carried a mutation/UV. This difference in T wave was more evident among the PKP2 mutation carriers.<sup>5</sup> Disease onset was slightly earlier among the small group of DSG2 carrier patients (including the one with an additional DSC2 mutation) compared to those with a PKP2 mutation in the TFC+ group (34 years vs 36 years; not significant. No other significant differences in any other parameters could be established.

### **Discussion**

In this multicenter Dutch study we have systematically evaluated a large number of ARVD/C patients, both clinically and genetically. We have screened three cardiac desmosomal genes (PKP2, DSG2 and DSC2) in 116 patients, of whom 57 were TFC+. PKP2 mutations were most prevalent in TFC+ patients, who comprised just under half of the ARVD/C patients (40%). Mutations in DSG2, DSC2 or both were found in 4, 1 and 1 of ARVD/C index patients, respectively (7%, 2%, 2%); this frequency is similar to reports published from other countries.<sup>12,14,17</sup> DSG2 mutations ranged between 3.5-17% in our probable ARVD/C patients and in those with 1 major or 2 minor criteria for ARVD/C. The DSC2 mutation frequencies in these groups were 7% and 0%, respectively. In approximately 50% of our TFC+ ARVD/C patients, we identified no mutation in any of the three desmosomal

genes screened, although large deletions or mutations in regulatory regions (promoter/intronic) cannot be excluded. Mutations in other genes related to ARVD/C are believed to be involved far less often and might add only a few more patients to our groups.<sup>4</sup>

In this study we have identified compound heterozygous or homozygous mutations in DSG2 in two unrelated patients. In addition, a third patient carried mutations in both DSG2 and DSC2. Interestingly, all three patients fall in the full blown ARVD/C group (TFC+). Moreover, in two patients from the TFC+ group, p.Arg46Gln (DSG2) occurred with p.Val158Gly on the same allele. Haplotype analysis confirmed a common founder (data not shown). Previously, p.Val158Gly has been reported in two families as potentially pathogenic,<sup>16</sup> however, we found p.Val158Gly in 1% of our control subjects, similar to the frequency reported by Posch et al.<sup>27</sup> Thus we cannot conclude at this stage whether the presence of this p.Val158Gly adds to the pathogenicity of the p.Arg46Gln mutation. Though previous studies detected compound heterozygous DSG2 mutations in two TFC+ ARVD/C patients,<sup>12,14</sup> the majority of the mutation carriers were heterozygous for a DSG2 mutation, unlike our ARVD/C patients.<sup>12,14</sup> But it should be noted that previous studies did not look for digenic mutations in their ARVD/C cohorts.<sup>5,7,9,10,12-16,19,20</sup> This suggests that the heterozygous missense mutations in the genes encoding desmosomal cadherins (DSG2/DSC2) are associated with a less fulminant phenotype. This is corroborated by the fact that we identified mutations in these genes predominantly in the probable ARVD/C patients or group of patients with 1 major or 2 minor criteria. However, this observation in small groups has to be confirmed in a larger series. Further, we have only identified missense mutations in DSG2 and in DSC2 in our Dutch cohort, whereas three published reports reported mainly missense mutations, as well as some truncating mutations, in these two genes in their ARVD/C cohorts.<sup>12,14,17</sup> Moreover, except for the family with the p.Val392Ile mutation, we found no significant predilection for left ventricle involvement among DSG2 mutation carriers in our study, as has been reported in a UK population by Syrris et al. 2007.<sup>17</sup>

Patient-40 (TFC+) and another unrelated patient (not shown in Table) harbored the identical mutation p.Val392Ile in DSG2, but their clinical presentation is quite variable. Patient-40 had full blown features of ARVD/C (TFC+) (Table 2), while the patient from the 2 minor and 1 major criteria group demonstrated a left-dominant arrhythmogenic cardiomyopathy. We think the difference in phenotype could be attributed to the fact that patient-40 (TFC+) harbored a second mutation, p.Leu732Val, in DSC2.

During this study, we also observed a frequent polymorphism, p.Glu713Lys (DSG2), equally prevalent among controls and the ARVD/C population (4%), which has also been described by Posch et al. as an innocent SNP.<sup>27</sup> However, suspicion about the pathogenicity of p.Val56Met (DSG2) should be verified as we did not detect p.Val56Met in controls in our study, in contrast to Posch et al.<sup>27</sup>

In DSC2, variant p.E896fsX5 was detected in three different families and in 4% of our control population. We therefore now consider this to be a non-pathogenic common variant in our population, although in a previous study it was considered pathogenic.<sup>16</sup>

As to the phenotype, we have observed predominance of negative T waves in V1-V3 among the mutation carriers, more specifically among the PKP2 mutation carriers. Turrini et al. (2001)<sup>29</sup> while retrospectively studying ECG parameters in various groups of ARVD/C patients, found negative T waves in precordial leads as a predictor of sudden cardiac death. Unfortunately they provided no genotype data for the ARVD/C patients in their study.<sup>28</sup> Another study from the Netherlands suggested that repolarization abnormalities in ARVD/C could facilitate the occurrence of ventricular arrhythmias in these patients.<sup>29</sup> We observed the significant predominance of negative T waves among individuals with the PKP2 mutation, which could be used to direct genetic screening.<sup>5</sup>

In conclusion, we found DSG2 and DSC2 mutations in approximately 10% of TFC+ patients in our Dutch ARVD/C cohort. We also identified mutations in these genes in 10% of probable ARVD/C cases. Interestingly, we found compound and digenic mutations exclusively in TFC+ patients, suggesting a dose effect of mutations correlating with disease severity, although larger studies are needed to confirm this observation.

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## References

1. Marcus FI, Fontaine G, Guiraudon G, Frank R, Laurenceau JL, Malergue S, Gros-gogeat Y. Right ventricular dysplasia. A report of 24 adult cases. *Circulation*. 1982; 65:384-98.
2. Corrado D, Basso C, Thiene G, McKenna WJ, Davies MJ, Fontaliran F, Nava A,



Silvestri F, Blomstrom-Lundqvist C, Wlodarska EK, Fontaine G, Camerini F. Spectrum of clinicopathologic manifestations of arrhythmogenic right ventricular cardiomyopathy/dysplasia: a multicenter study. *J Am Coll Cardiol.* 1997; 30:1512-20.

3. Sen-Chowdhry S, Syrris P, Ward D, Asimaki A, Sevdalis E, McKenna WJ. Clinical and genetic characterization of families with arrhythmogenic right ventricular dysplasia/cardiomyopathy provides novel insights into patterns of disease expression. *Circulation.* 2007;115:1710-20.

4. Sen-Chowdhry S, Syrris P, McKenna WJ. Role of genetic analysis in the management of patients with arrhythmogenic right ventricular dysplasia/cardiomyopathy. *J Am Coll Cardiol.* 2007;50:1813-21.

5. van Tintelen JP, Entius MM, Bhuiyan ZA, Jongbloed R, Wiesfeld AC, Wilde AA, van der Smagt J, Boven LG, Mannens MM, van Langen IM, Hofstra RM, Otterspoor LC, Doevendans PA, Rodriguez LM, van Gelder IC, Hauer RN. Plakophilin-2 mutations are the major determinant of familial arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Circulation.* 2006;113:1650-8.

6. Rampazzo A, Nava A, Malacrida S, Beffagna G, Bauce B, Rossi V, Zimbello R, Simionati B, Basso C, Thiene G, Towbin JA, Danieli GA. Mutation in human desmoplakin domain binding to plakoglobin causes a dominant form of arrhythmogenic right ventricular cardiomyopathy. *Am J Hum Genet.* 2002;71:1200-6.

7. Gerull B, Heuser A, Wichter T, Paul M, Basson CT, McDermott DA, Lerman BB, Markowitz SM, Ellinor PT, MacRae CA, Peters S, Grossmann KS, Drenckhahn J, Michely B, Sasse-Klaassen S, Birchmeier W, Dietz R, Breithardt G, Schulze-Bahr E, Thierfelder L. Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy. *Nat Genet.* 2004;36:1162-4.

8. Bauce B, Basso C, Rampazzo A, Beffagna G, Daliento L, Frigo G, Malacrida S, Settimo L, Danieli G, Thiene G, Nava A. Clinical profile of four families with arrhythmogenic right ventricular cardiomyopathy caused by dominant desmoplakin mutations. *Eur Heart J.* 2005;26:1666-75.

9. Kannankeril PJ, Bhuiyan ZA, Darbar D, Mannens MM, Wilde AA, Roden DM. Arrhythmogenic right ventricular cardiomyopathy due to a novel plakophilin 2 mutation: wide spectrum of disease in mutation carriers within a family. *Heart Rhythm.* 2006;3: 939-44.

10. Dalal D, Molin LH, Piccini J, Tichnell C, James C, Bomma C, Prakasa K, Towbin JA, Marcus FI, Spevak PJ, Bluemke DA, Abraham T, Russell SD, Calkins H, Judge DP. Clinical features of arrhythmogenic right ventricular dysplasia/cardiomyopathy associated with mutations in plakophilin-2. *Circulation.* 2006;113:1641-9.

11. Yang Z, Bowles NE, Scherer SE, Taylor MD, Kearney DL, Ge S, Nadvoretzkiy

- VV, Defreitas G, Carabello B, Brandon LI, Godsel LM, Green KJ, Saffitz JE, Li H, Danieli GA, Calkins H, Marcus F, Towbin JA. Desmosomal Dysfunction due to Mutations in Desmoplakin Causes Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy. *Circ Res.* 2006;99:646-55.
12. Awad MM, Dalal D, Cho E, Amat-Alarcon N, James C, Tichnell C, Tucker A, Russell SD, Bluemke DA, Dietz HC, Calkins H, Judge DP. DSG2 mutations contribute to arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Am J Hum Genet.* 2006;79:136-42.
13. Awad MM, Dalal D, Tichnell C, James C, Tucker A, Abraham T, Spevak PJ, Calkins H, Judge DP. Recessive arrhythmogenic right ventricular dysplasia due to novel cryptic splice mutation in PKP2. *Hum Mutat.* 2006;27:1157.
14. Pilichou K, Nava A, Basso C, Beffagna G, Bauce B, Lorenzon A, Frigo G, Vettori A, Valente M, Towbin J, Thiene G, Danieli GA, Rampazzo A. Mutations in desmoglein-2 gene are associated with arrhythmogenic right ventricular cardiomyopathy. *Circulation.* 2006;113:1171-9.
15. Syrris P, Ward D, Asimaki A, Sen-Chowdhry S, Ebrahim HY, Evans A, Hitomi N, Norman M, Pantazis A, Shaw AL, Elliott PM, McKenna WJ. Clinical expression of plakophilin-2 mutations in familial arrhythmogenic right ventricular cardiomyopathy. *Circulation.* 2006;113:356-64.
16. Syrris P, Ward D, Evans A, Asimaki A, Gandjbakhch E, Sen-Chowdhry S, McKenna WJ. Arrhythmogenic right ventricular dysplasia/cardiomyopathy associated with mutations in the desmosomal gene desmocollin-2. *Am J Hum Genet.* 2006;79:978-84.
17. Syrris P, Ward D, Asimaki A, Evans A, Sen-Chowdhry S, Hughes SE, McKenna WJ. Desmoglein-2 mutations in arrhythmogenic right ventricular cardiomyopathy: a genotype-phenotype characterization of familial disease. *Eur Heart J.* 2007; 28:581-8.
18. Protonotarios N, Tsatsopoulou A, Anastasakis A, Sevdalis E, McKoy G, Stratos K, Gatzoulis K, Tentolouris K, Spiliopoulou C, Panagiotakos D, McKenna W, Toutouzas P. Genotype-phenotype assessment in autosomal recessive arrhythmogenic right ventricular cardiomyopathy (Naxos disease) caused by a deletion in plakoglobin. *J Am Coll Cardiol.* 2001;38:1477-84.
19. Heuser A, Plovie ER, Ellinor PT, Grossmann KS, Shin JT, Wichter T, Basson CT, Lerman BB, Sasse-Klaassen S, Thierfelder L, MacRae CA, Gerull B. Mutant desmocollin-2 causes arrhythmogenic right ventricular cardiomyopathy. *Am J Hum Genet.* 2006;79:1081-8.
20. Beffagna G, De Bortoli M, Nava A, Salamon M, Lorenzon A, Zacco M, Mancuso L, Sigalotti L, Bauce B, Occhi G, Basso C, Lanfranchi G, Towbin JA, Thiene G, Danieli GA, Rampazzo A. Missense mutations in desmocollin-2 N-terminus, associated with

arrhythmogenic right ventricular cardiomyopathy, affect intracellular localization of desmocollin-2 in vitro. *BMC Med Genet.* 2007;8:65.

21. McKoy G, Protonotarios N, Crosby A, Tsatsopoulou A, Anastasakis A, Coonar A, Norman M, Baboonian C, Jeffery S, McKenna WJ. Identification of a deletion in plakoglobin in arrhythmogenic right ventricular cardiomyopathy with palmoplantar keratoderma and woolly hair (Naxos disease). *Lancet.* 2000;355:2119-24.
22. Norman M, Simpson M, Mogensen J, Shaw A, Hughes S, Syrris P, Sen-Chowdhry S, Rowland E, Crosby A, McKenna WJ. Novel mutation in desmoplakin causes arrhythmogenic left ventricular cardiomyopathy. *Circulation.* 2005;112:636-42.
23. Asimaki A, Syrris P, Wichter T, Matthias P, Saffitz JE, McKenna WJ. A novel dominant mutation in plakoglobin causes arrhythmogenic right ventricular cardiomyopathy. *Am J Hum Genet.* 2007;81:964-73.
24. Merner ND, Hodgkinson KA, Haywood AF, Connors S, French VM, Drenckhahn JD, Kupprion C, Ramadanova K, Thierfelder L, McKenna W, Gallagher B, Morris-Larkin L, Bassett AS, Parfrey PS, Young TL. Arrhythmogenic Right Ventricular Cardiomyopathy Type 5 is a fully penetrant, lethal arrhythmic disorder caused by a missense mutation in the TMEM43 gene. *Am J Hum Genet* 2008;82:809-21.
25. McKenna WJ, Thiene G, Nava A, et al. Diagnosis of arrhythmogenic right ventricular dysplasia/cardiomyopathy. Task Force of the Working Group Myocardial and Pericardial Disease of the European Society of Cardiology and of the Scientific Council on Cardiomyopathies of the International Society and Federation of Cardiology. *Br Heart J.* 1994;71:215-8.
26. Goldgar DE, Easton DF, Deffenbaugh AM, Monteiro AN, Tavtigian SV, Couch FJ; Breast Cancer Information Core (BIC) Steering Committee. Integrated evaluation of DNA sequence variants of unknown clinical significance: application to BRCA1 and BRCA2. *Am J Hum Genet.* 2004;75:535-44.
27. Posch MG, Posch MJ, Geier C, Erdmann B, Mueller W, Richter A, Volker R, Pankuweit S, Maisch B, Perrot A, Buttgerit J, Dietz R, Haverkamp W, Özcelik C. A missense variant in desmoglein-2 predisposes to dilated cardiomyopathy. *Mol Genet Metab.* 2008;95:74-80.
28. Lahtinen AM, Lehtonen A, Kaartinen M, Toivonen L, Swan H, Widén E, Lehtonen E, Lehto VP, Kontula K. Plakophilin-2 missense mutations in arrhythmogenic right ventricular cardiomyopathy. *Int J Cardiol.* 2008;126:92-100.
29. Turrini P, Corrado D, Basso C, Nava A, Bauce B, Thiene G. Dispersion of ventricular depolarization-repolarization: a noninvasive marker for risk stratification in arrhythmogenic right ventricular cardiomyopathy. *Circulation.* 2001;103:3075-80.
30. Peeters HA, SippensGroenewegen A, Schoonderwoerd BA, Wever EF, Grim-

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bergen CA, Hauer RN, Rohles de Medina EO. Body-surface QRST integral mapping. Arrhythmogenic right ventricular dysplasia versus idiopathic right ventricular tachycardia. *Circulation*. 1997;95:2668-76.